

CLAIMS

What is claimed is:

1. A method of identifying a cDNA construct wherein the cDNA construct expresses a tagged polypeptide having a biochemical activity of interest comprising the steps of:
- 5 a) preparing a tagged cDNA expression library comprising bacterial cells comprising tagged cDNA plasmid constructs;
- b) culturing the bacterial cells of step a) to produce clones wherein each clone corresponds to a single tagged cDNA construct;
- 10 c) arraying the individual bacterial clones;
- d) pooling a predetermined number of arrayed clones and isolating plasmid DNA from them;
- e) transfecting suitable mammalian host cells with the pooled plasmid clones and maintaining the transfected cells under conditions suitable for the expression of the tagged cDNA construct, thereby producing tagged polypeptides;
- 15 f) assaying the expressed tagged polypeptides for a biochemical activity of interest; and
- identifying a pool of clones comprising a cDNA construct encoding the tagged polypeptide having the biochemical activity of interest.
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2. The method of Claim 1 wherein steps d) through f) are repeated until a single cDNA construct expressing a tagged polypeptide having the biochemical activity of interest is identified.
3. The method of Claim 1 wherein the tag is selected from the group consisting of:
- 25 GST-, Myc-, HA-, FLAG- and His-.

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4. The method of Claim 1 wherein preparing the tagged cDNA expression library of step a) comprises the steps of:
- i) obtaining double-stranded cDNA from cells expressing a polypeptide with the biochemical activity of interest;
 - 5 ii) ligating the cDNA into an expression vector wherein the expression vector comprises a coding region for a tag operably linked to a promoter to produce a tagged cDNA construct; and
 - iii) transforming competent bacterial cells with the tagged cDNA construct of step ii).
- 10 5. The method of Claim 4 wherein the tagged cDNA library comprises cDNA constructs having specific protein motifs that have been selected by polymerase chain reaction.
6. The method of Claim 4 wherein the promoter in step ii) is EF-1 α .
7. The method of Claim 1 wherein the mammalian host cells used in step e) are 293
15 T fibroblast cells.
8. The method of Claim 1 wherein the biochemical activity of interest is selected from the group consisting of:
- a) acting as a substrate for a specific enzyme;
 - b) being a specific enzyme;
 - 20 c) interacting with specific antibodies;
 - d) forming specific protein-protein associations;
 - e) forming specific protein-nucleic acid associations;
 - f) interacting specifically with any biological element or compound;
 - g) possessing cell biological activity such as growth, differentiation,
25 apoptosis, vascularization, motility or morphological change promoting or inhibiting ;

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- h) undergoing specific post-translational modifications (phosphorylation, glycosylation, ubiquitination, acetylation, proteolytic cleavage, *etc.*) in mammalian cells;
- i) possessing any of the activities in a-h only in response to a specific stimuli in mammalian cells.
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9. The method of Claim 1 wherein step d) each pool of clones comprises from about 2 to about 1000 clones.
10. A pool of clones comprising a cDNA construct encoding a tagged polypeptide having a biochemical activity of interest identified by the method of Claim 1.
- 10 11. A cDNA construct encoding a tagged polypeptide having a biochemical activity of interest identified by the method of Claim 1 or 2.
12. The method of Claim 1 wherein more than one expression library is prepared and each expression library comprises a different cell type wherein the cells are stimulated with a specific stimulus.

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